Evaluation of chemoenhancing and chemoprotective efficacy of 3',3'-diindolylmethane in combination with doxorubicin against triple-negative breast cancer

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ABSTRACT

Triple-negative breast cancer (TNBC) is a type of breast cancer that is very aggressive and has a poor prognosis. It is defined by the absence of oestrogen receptor (ER), progesterone receptor (PR), and HER2 expression. This receptor negativity renders TNBC unresponsive to targeted hormonal or HER2-based therapies, leaving chemotherapy as the primary treatment option. Doxorubicin (DOX), an anthracycline chemotherapeutic agent, remains a widely used drug against TNBC, but its clinical application is limited by systemic toxicities, particularly cardiotoxicity, and the emergence of chemoresistance. Therefore, there is a need for combination strategies that can improve therapeutic efficacy while reducing adverse effects.

This study evaluates the chemoenhancing and chemoprotective potential of 3',3'-diindolylmethane (DIM), a natural bioactive compound derived from cruciferous vegetables, in combination with DOX in both in vitro and in vivo TNBC models.

In vitro studies were conducted on the MDA-MB-231 cell line. The combination of DIM and DOX significantly inhibited cell viability (MTT assay), induced G2/M cell cycle arrest (flow cytometry), and enhanced apoptosis, as demonstrated by AO/EtBr staining and Annexin V/PI assays. Mechanistic insights revealed mitochondrial membrane depolarization (JC-1 assay), cytochrome-c release, and increased expression of pro-apoptotic markers such as BAX, Caspase-3, and APAF-1, with concurrent downregulation of BCL-2, confirmed by western blotting and immunocytochemistry. Combination treatment also made it harder for cells to migrate (scratch assay) and form colonies (clonogenic assay).

In vivo experiments using BALB/c mice with 4T1 TNBC tumours showed that the DIM+DOX combination significantly reduced tumour volume and improved histopathological features. Notably, DIM co-administration mitigated DOX-induced cardiotoxic effects and histopathological abnormalities in bone marrow.

Overall, the study highlights DIM's dual role as a chemoenhancer and chemoprotectant. These findings support the potential utility of DIM as an effective adjuvant in DOX-based chemotherapy regimens for triple-negative breast cancer, offering improved therapeutic outcomes with reduced systemic toxicity.

Keywords: Triple-negative breast cancer (TNBC), 3',3'-Diindolylmethane (DIM), Doxorubicin (DOX), Apoptosis, Chemoprotection, Combination therapy.